Amendments to the Drawings

Please delete original drawing sheet 1/1 and enter the enclosed replacement sheet in its place. The only amendment that has been made is that the title "Figure 1" has been added. Thus, the amendments do not add new matter to the application.

Remarks

I. Status of the Application and Claims

As originally filed, the present application had a total of 12 claims. All of these have now been cancelled and replaced with new claims 13-22.

II. The Amendments

New claim 13, defines the galactose-proton symporter as having the amino acid sequence of SEQ ID NO:4. Support for this amendment may be found on page 10 of the application, lines 5-11. A list of carbon sources used in the process has been added. Support for this list may be found on page 7 of the application, lines 27 and 28. Support for reference to an endogenous sequence in claim 13 may be found on page 10 of the application, lines 13-18.

Support for new claim 16, may be found on page 10 of the application, lines 5-11.

Support for new claims 18-20 may be found in original claims 2 and 3.

Support for new claims 21 and 22 may be found in original claims 8 and 9.

The amendments do not add new matter to the application and their entry is therefore respectfully requested.

III. Objections to the Specification and Drawings

On pages 3 and 4 of the Office Action, the Examiner objects to the title and abstract of the application as well as to the only drawing present.

In response, Applicant has amended both the title and abstract. In addition a replacement drawing has been submitted herewith in which the only amendment that has been made is that the title "Figure 1" has been added. Applicant believes that these amendments should be sufficient to overcome the objections made and it is respectfully requested that they be withdrawn.

The Rejections

I. Rejection of Claims Under 35 U.S.C. § 112, Second Paragraph

On pages 4 and 5 of the Office Action, the Examiner makes two rejections under 35 USC § 112, second paragraph. In item 7, claims 1-9 are rejected based upon the allegation that the phrases "the galP gene" and "another nucleotide sequence coding for galP" make the claims indefinite. However, since the combination of phrases objected to is no longer in the amended claims, it is respectfully submitted that this rejection has been obviated.

In item 8, claims 4 and 5 are rejected based upon the allegation that the phrases "the biosynthesis pathway" and "metabolic pathway" are indefinite. However, since no counterpart to either claim 4 or claim 5 is in the amended claims, it is submitted that this rejection has been obviated.

II. Rejection of Claims Under 35 U.S.C. § 112, First Paragraph

On pages 5-10 of the Office Action, the Examiner makes three rejections under the first paragraph of 35 USC § 112. These are set forth in items 9-11 and Applicant responds to each below.

A. Response to Allegations in Item 9

In item 9, claims 1-9 are rejected as failing to meet the written description requirement of patentability. This rejection is based upon the allegation that the claims fail to provide a structural definition for the galP gene or for the protein it encodes.

In response, Applicant has amended claims so that they are all now limited to the specific amino acid or nucleotide sequences as set forth in either SEQ ID NO:3 or SEQ ID NO:4. In light of these amendments, Applicant submits that the Examiner's rejection has been overcome.

B. Response to Allegations in Item 10

In item 10, the Examiner rejects claims 1-5 and 8-9 based upon the written description requirement. The main new allegation made is that the claims encompass methods of increasing galP gene product activity by altering the catalytic properties of the protein and a description of how this could be accomplished is not provided. In response, claims have been

limited to bacteria with "an overexpressed endogenous DNA sequence encoding the galactose-proton symporter protein." Thus, methods in which catalytic activity is altered are no longer part of the claims.

Similar allegations are made with respect to claims 8 and 9 but, again, the counterpart amended claims (*i.e.*, claims 21 and 22, are limited to changes in gene expression and do not encompass changing catalytic activity.

C. Response to Allegations in Item 11

In item 11, the Examiner essentially repeats the allegations of item 10 but rejects claims on enablement rather than written description grounds. As previously discussed, the amended claims no longer include methods in which the catalytic properties of a protein are altered. Thus, the present enablement rejection has been overcome.

III. Rejection of Claims Under 35 U.S.C. §§ 102

A. Rejection of Claims Based Upon Valle *et al.*

On pages 11 and 12 of the Office Action, the Examiner rejects claims 1-5 under 35 USC §102 as being anticipated by Valle *et al.* (US 2002/015521). It is alleged that the reference discloses E. coli that have increased levels of galP and that these bacteria produce increased amounts of amino acids. The Examiner argues that this is sufficient to anticipate claim 1 and that the limitations added in claims 2-5 are either taught or inherent in the reference.

Applicant respectfully traverses this rejection for the claims as amended herein.

First, it is important to understand that Applicant believes that the novelty and nonobviousness of the claimed invention lies in the relationship between increased galP activity and increased amino acid production by bacteria. The gene itself was known in the art prior to the filing of the application and methods of engineering cells to increase gene expression are routine in the art of molecular biology. However, the metabolic pathways involved in galactose metabolism are complex and, despite the Valle reference, there was no way to predict that there would be a direct correlation between galP expression and amino acid production prior to the time that the present application was filed. In order to appreciate

why this is so, it is important to understand a few points about the relevant metabolic pathways.

The PEP-dependent phosphotransferase (PTS) pathway is involved in promoting the transport of glucose into bacterial cells. In doing this, the pathway utilizes PEP and thereby makes less of this compound available for use in the synthesis of amino acids.[1] Knowing this, the Valle inventors postulated that they might be able to increase amino acid synthesis in bacteria if they: a) blocked the PTS pathway so that more PEP was available; and b) then selected bacteria carrying mutations that allowed them to compensate for the loss of PTS-stimulated glucose transport. This is described clearly in paragraph [0005] of the reference (page 1, second column). It should be noted that improvements in amino acid production are attributed to the inactivation of the PTS pathway. The additional mutations are disclosed as allowing the cells to make up for the loss of glucose, not to promote amino acid production per se.

The strategy of the Valle inventors was successful; they were able to identify PTS^- cells that produced increased amounts of amino acids during fermentation. They then conducted further experiments that suggested that the mutations that compensated for the loss glucose had the effect of increasing galP activity. In other words, the cells were compensating for the loss of glucose transport by the PTS pathway by increasing transport using a secondary, galP, pathway. However, the reference never suggests that increasing galP activity in normal, *i.e.*, PTS positive, bacteria would have the same effect (either with respect to providing cells with an energy source or with respect to amino acid production). In fact, Valle expressly suggests that galP-based transport is of no physiological relevance unless the PTS pathway is blocked. This can be seen in paragraph [0034] (page 4) which reads as follows:

> The mutants characterized in this work apparently carry more than one mutation and need galactose permease (galP) activity to give the described phenotypes. It is known that the best substrate for galP is D-glucose. In normal conditions (i.e., with a functional PTS) this is not of physiological relevance, especially because PTS is responsible for inducer exclusion[2] and the galactose

[1] This is illustrated in the top part of Figure 1 in the Valle reference.

[2] The term "inducer exclusion" refers to the inhibition by glucose of the uptake of other sources of carbon by cells. Valle is saying that when the PTS pathway is functioning, glucose acts to promote its further uptake and inhibits the expression of genes involved in galactose uptake and utilization. It is only after the PTS pathway is blocked that this inhibitory effect is lost and that mutations increasing the activity galactose permease (which is capable of transporting both glucose and

> regulon is not induced, even if galactose is present in the medium. However, the deletion of ptsH1crr creates a new situation, inducer exclusion effect is lost. Under these circumstance, any mutation that turns on the galP gene (or any other transporter gene which product could transport glucose) should produce cells that can utilize glucose. However, the degree of glucose utilization will depend on the specificity, level, efficiency, etc., of the transporter.[3]

In rejecting claims. the Examiner points specifically to paragraphs 0057 and 0058 on page 6 of Valle. However, Applicant submits that a careful reading of these paragraphs shows that they only teach that mutations increasing galP activity increase amino acid production when bacteria also carry mutations inactivating the PTS pathway. The mutations compensate for the loss in PTS-induced glucose transport (see page 9, left column, line 8-10 and page 6, left column, line 9-11 from the bottom) but there is no suggestion that they contribute directly to increased amino acid production or that they would do so if the PTS pathway was still active.

B. Rejection of Claims Based Upon Grossiord *et al.*

On pages 12 and 13 of the Office Action, the Examiner rejects claims 1-2 and 6-7 under 35 USC §102 as being anticipated by Grossiord *et al.* (*J. Bacteriol. 185*:870-8878 (2003)). It is alleged that the reference teaches increasing the copy number of the galP gene in E. coli by transfecting the bacteria with plasmids. It is also argued that the reference teaches the inherent production of threonine by the cells as evidenced by the fermentation and DNA isolation steps described therein. The Examiner also cites Ravnikar, *et al.* (*J. Bacteriol. 169*:4716-4761 (2003)), apparently as verifying that genes transfected in the manner described by Grossiord would be constitutively expressed.

Applicant respectfully traverses this rejection for the claims as amended herein.

It is respectfully submitted that the references cited do not teach that increasing galP expression will lead to an increase in amino acid production or suggest that the engineered bacteria should be used in fermentations in which L-amino acids are isolated. The fact that the recombinant galP described in Grossiord is constitutively expressed or that the bacteria

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galactose) become important.

inherently produce threonine clearly does not mean that production has increased as a result of the increased levels of galP or suggest that the recombinantly engineered cells should be used in amino acid production methods. Thus, Applicant submits that this reference is not anticipatory with respect to the claims as amended herein.

In addition, Applicant submits that Grossiord teaches away from the use of the specific carbon sources now recited in claim 13 for amino acid production. The reference reports that when glucose is present as a carbon source, glucokinase activity and galactose uptake are very low (page 874, Table 4). In order to get high uptake and activity galactose must be present without glucose. Thus, according to Grossiord's teachings, it would make little sense to attempt to increase amino acid production by increasing galactose permease activity unless bacteria are grown under conditions of high galactose and low glucose. Since galactose is not an option in claim 13, the claimed process is both novel and non-obvious.

IV. Rejection of Claims Under 35 U.S.C. §§ 103

On pages 13-15 of the Office Action, claims 8 and 9 are rejected under 35 USC §103. Claim 8 is rejected as being obvious based upon Valle *et al.* in combination with Debabov *et al.* (US 6,132,999). Claim 9 is rejected based upon Valle *et al.* in combination with a second reference by Debabov *et al.* (US 5,705,371). In each case, the Valle reference is cited for the reasons stated above. Debabov '999 reference is cited as teaching that increased amino acid production can be achieved by increasing expression of the ABC operon and the Debabov '371 reference is cited as teaching that amino acid production can be increased by attenuating the expression of the tdh gene.

Applicant submits that each of the rejections under §103 depend upon the Valle reference disclosing that bacteria can be engineered to increase amino acid production by increasing the expression of galP. However, as discussed in connection with the rejection of claims under §102, Valle's teachings are limited to teaching that increased expression of galP leads to compensates for a loss of glucose transport in bacteria in which the PTS pathway has been inactivated. It does not teach that overexpression of galP directly contributes to increased amino acid production or that it would have any effect at all when the PTS pathway

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[3] Citations have been omitted in the quote.

has not been inactivated. In light of this, Applicant submits that the rejection of claims under §103 can not be sustained.

Conclusion

In light of the amendments and discussion above, Applicant believes that all of the Examiner's rejections have been overcome. It is therefore respectfully requested that these rejections be withdrawn and that the claims now pending be allowed. Early notice to this effect is earnestly solicited.

If, in the opinion of the Examiner, a phone call would help to expedite the prosecution of this application, the Examiner is invited to call Applicant's undersigned attorney at (202) 419-7013.

Respectfully submitted,

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